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(54) PRODUCTION OF TRIGLYCERIDE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for producing a new triglyceride in which a structural lipid considered as a human breast milk type triglyceride structure, i.e., the 2-position of the triglyceride is a 16-18C saturated fatty acid and at least one of unsaturated fatty acids bound to the 1- and the 3-positions are ω3-, ω 6- and/or ω 9-based unsaturated fatty acids.
SOLUTION: A triglyceride having ≤45°C melting point in which a fatty acid at the 2-position is a 16-18C saturated fatty acid and fatty acids at the 1- and the 3-positions are once converted into medium-chain fatty acids is used as a raw material or the process is passed through the resultant triglyeride as an intermediate to thereby produce the objective triglyceride when making a lipase capable of specifically acting on ester bonds at the 1- and the 3- positions act on a glyceride in which the 16-18C saturated fatty acid is bound to the 2-position and producing the triglyceride in which the fatty acids at the 1- and the 3-poditions are converted into ω 3-, ω 6- and/or ω 9-based unsaturated fatty acids according to a transesterification.

CLAIMS

[Claim(s)]

[Claim 1]Fatty acid of the 2nd place of triglyceride is saturated fatty acid of the carbon numbers 16-18, It is the method of manufacturing triglyceride of fatty acid of 1 and the 3rd place whose one is the unsaturated fatty acid of omega3, omega6, or omega9 system at least, the melting point 1 and whose fatty acid of the 3rd place fatty acid of the 2nd place of triglyceride is saturated fatty acid of the carbon numbers 16-18, and are medium chain fatty acid -- triglyceride 45 ** or less -- omega3, omega6, or omega -- under existence of unsaturated fatty acid or its ester 9 system, A manufacturing method of the triglyceride concerned making specific lipase act the 1 or 3rd place, and obtaining target triglyceride by an ester exchange reaction.

[Claim 2]Triglyceride to manufacture is [fatty acid of the 2nd place of triglyceride] saturated fatty acid of the carbon numbers 16-18, A manufacturing method of the triglyceride according to claim 1 which one side of fatty acid of 1 and the 3rd place is the unsaturated fatty acid of omega3, omega6, or omega9 system, and is the unsaturated fatty acid of omega3 as the unsaturated fatty acid concerned also with same another side, omega6, or omega9 system.

[Claim 3]Triglyceride to manufacture is [fatty acid of the 2nd place of triglyceride] saturated fatty acid of the carbon numbers 16-18, A manufacturing method of the triglyceride according to claim 1 which one side of fatty acid of 1 and the 3rd place is the unsaturated fatty acid of omega3, omega6, or omega9 system, and is the unsaturated fatty acid of omega3, omega6, or omega9 system in which another side differs from the unsaturated fatty acid concerned.

[Claim 4]A manufacturing method of the triglyceride according to claim 1 whose another side fatty acid of the 2nd place of triglyceride is [triglyceride to manufacture] saturated fatty acid of the carbon numbers 16-18, one side of fatty acid of 1 and the 3rd place is the unsaturated fatty acid of omega3, omega6, or omega9 system, and is the saturated fatty acid of the carbon numbers 4-18.

[Claim 5]Unsaturated fatty acid of omega3, omega6, and/or omega9 system, 9, 12, and 15-octadecatrienoic acid . (alpha- linolenic acid) 18:3 and omega3 6 and 9, 12 and 15-octadeca tetraenoic acid (steer RIDON acid). 18:4 and omega3 11, 14, and 17-. Eicosatrienoic acid (******- alpha-linoleic acid). 20:3 and omega3 8, 11, 14, the 17-eicosatetraenoic acid 20:4, and omega3 5, 8, 11, 14, 17-eicosapentaenoic acid 20:5, and omega3 7, 10, 13, 16, and 19-docosapentaenoic acid . 22:5 and omega3 4, 7, 10, and 13, 16, the 19-docosahexaenoic acid 22:6, and omega3. 9 and 12-octadecadienoic acid (linolic acid). 18:2 and omega6 6, 9, and 12- octadecatrienoic acid . (Gamma-linolenic acid) 18:3 and omega6 8, 11, 14-eicosatrienoic acid (******- .) The gamma- linolenic acid 20:3 and omega6 5, 8, 11, 14-eicosatetraenoic acid (arachidonic acid)

20:4 and omega6 7, 10, 13,16-docosatetraenoic acid 22:4, and omega6 4, 7, 10, 13, 16-docosapentaenoic acid 22:5, omega66, 9-octa DEKAJI. Enoic-acid 18: 2 and omega9 8, 11-eicosadienoic acid 20:2, and omega9 5, 8, 11- eicosatrienoic acid (mead acid) A manufacturing method of triglyceride given in any 1 paragraph of claims 1 thru/or 4 which is the unsaturated fatty acid chosen from a group which consists of 20:3 and omega9.

[Claim 6]Triglyceride which saturated fatty acid of the carbon numbers 16-18 combined with the 2nd place used for an ester exchange reaction, omega3, omega6, and/or omega -- it being from microorganism [which has the capability to produce unsaturated fatty acid as constituent fatty acids of triglyceride 9 system], and, A manufacturing method of triglyceride given in any 1 paragraph of claims 1 thru/or 5 being triglyceride which saturated fatty acid of the carbon numbers 16-18 combined with the 2nd place of the triglyceride concerned, and unsaturated fatty acid of omega3, omega6, or omega9 system has combined with 1 and the 3rd place.

[Claim 7]A manufacturing method of triglyceride given in any 1 paragraph of claims 1 thru/or 5, wherein unsaturated fatty acid of omega3 and omega6 which are added for an ester exchange reaction, or omega9 system is a hydrolysis mixture of triglyceride which uses unsaturated fatty acid of omega3, omega6, or omega9 system as constituent fatty acids.

[Claim 8]A manufacturing method of triglyceride given in any 1 paragraph of claims 1 thru/or 5 whose fatty acid of the 2nd place of triglyceride is pulmitic acid or stearic acid.

[Claim 9]A manufacturing method of triglyceride given in any 1 paragraph of claims 1 thru/or 8 performing an ester exchange reaction by the system of reaction which does not use an organic solvent.

[Claim 10]A manufacturing method of triglyceride given in any 1 paragraph of claims 1 thru/or 9 performing an ester exchange reaction for reaction temperature below 45 **.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the manufacturing method of new triglyceride, and relates to the manufacturing method of the triglyceride which has the saturated fatty acid of the carbon numbers 16-18 especially in the 2nd place of triglyceride, and has the unsaturated fatty acid of omega3, omega6, and/or omega9 system in either [at least] 1 or the 3rd place.

[0002]

[Description of the Prior Art]The great portion of lipid which we are taking in is neutral fat, and it is a mixture of the triglyceride in which 1 of triglyceride, 2, and various fatty acid carried out the ester bond to the 3rd place. And it is pointed out by the difference in the connecting position of fatty acid that the physiology activity differs, and the lipid (structure lipid) which combined specific fatty acid with the position it was decided that triglyceride would be attracts attention especially these days.

[0003]for example, JP,4-12920,B **** -- good triglyceride of the digestion nature which fatty acid of the carbon numbers 8-14 combined with the 2nd place of triglyceride, and fatty acid whose carbon number is 18 or more combined with 1 and the 3rd place is indicated. 2 - JP,5-87497,B since monoglyceride is considered to be a gestalt which tended to be absorbed by people's living body ****. omega3 which has a physiological function in the 2nd place, or omega -- the triglyceride which combined the higher unsaturated fatty acid 6 system, and combined the saturated fatty acid easily hydrolyzed into 1 and the 3rd place with the enzyme of an alimentary canal is indicated.

[0004]On the other hand, if their eyes are turned to the physiological function of fatty acid, arachidonic acid and docosahexaenoic acid attract attention in recent years. These fatty acid is contained in mother's milk. A report ("Advances in Polyunsaturated Fatty Acid Research", Elsevier Science Publishers, 1993, pp.261-264) that it is useful for a suckling's growth, There is a report (Proc. Natl. Acad. Sci. USA, 90, 1073-1077 (1993), Lancet, 344, and 1319-1322 (1994)) of being important for embryonic height or cerebral growth.

[0005]And a recommended intake is released from some public institutions (premature baby: docosahexaenoic acid 20 mg/kg weight / [the arachidonic acid 60, the docosahexaenoic acid 40; normal-child:arachidonic acid 20, and] day (WHO-FAO (1994)).). In the several countries in Europe, the modified milk for premature babies which blended as triglyceride the arachidonic acid which already combined with docosahexaenoic acid and carried out fermentation production is marketed. However, it is not taken into

consideration about the connecting position of the arachidonic acid of triglyceride, and/or docosahexaenoic acid applied to modified milk.

[0006]The triglyceride structure in people's mother's milk has a high rate which pulmitic acid (16:0) combines with the 2nd place of triglyceride, 1. To the 3rd place, and a higher unsaturated fatty acid. . Or it is thought that the rate which medium chain fatty acid combines is high. (Christie, W.W. (1986) The Positional Distribution of Fatty Acids in Triglycerids. Analysis of Oils and Fats in (Hamilton,) R.J., and Russell, J.B., eds.pp. 313-339, Elsevier Applied Science, and London.

[0007]On the other hand, the structure of the arachidonic acid content triglyceride produced by the fermenting method added to modified milk in order to bring the above-mentioned fatty acid composition close to the presentation of mother's milk, Saturated fatty acid including pulmitic acid combines with 1 and the 3rd place, The rate combined with the 2nd place unsaturated fatty acid highly (J. J. Myher, A. Kuksis, K. Geher, P.W. Park, and D.A Diersen-Schade, Lipids 31, and 207-215 (1996)), It differed from what is considered to be people's mother's milk type clearly. Therefore, development of the structure lipid by which structure is checked clearly which the carbon number combined with the 2nd place (the structure lipid considered to be people's mother's milk type triglyceride structure, i.e., triglyceride), and a higher unsaturated fatty acid or medium chain fatty acid combined with the saturated fatty acid of 16-18, 1, and the 3rd place is desired strongly.

[0008]

[Problem(s) to be Solved by the Invention]Therefore, the structure lipid by which this invention is considered to be a Homo sapiens mother's milk type triglyceride structure, That is, the 2nd place of triglyceride is [a carbon number] the saturated fatty acid of 16-18, the unsaturated fatty acid combined with 1 and the 3rd place -- at least -- one -- omega3, omega6, or omega -- new triglyceride which is unsaturated fatty acid 9 system. or -- the 2nd place of triglyceride is [a carbon number] the saturated fatty acid of 16-18, and either 1 or the 3rd place is [a carbon number] the saturated fatty acid of 4-18 -- another side -- omega3, omega6, or omega -- it is going to provide the manufacturing method of new triglyceride which is unsaturated fatty acid 9 system.

[0009]

[Means for Solving the Problem]A method of manufacturing triglyceride which fatty acid of the carbon numbers 8-14 combined with the 2nd place of triglyceride by 1 and an ester exchange reaction using specific lipase the 3rd place, and fatty acid whose carbon number is 18 or more combined with 1 and the 3rd place is above-mentioned JP,4-12920,B. It is indicated. However, in order for fatty acid of the 2nd place to use as a raw material triglyceride in which a carbon number consists of saturated fatty acid of the carbon numbers 16-18 which increased further and at least for 1 and 3 to perform an ester exchange reaction with unsaturated fatty acid of omega3, omega6, or omega9 system using specific lipase, reaction temperature must be not less than 50 **. This reaction is a reaction which used immobilized enzyme, and, in a carbon number, saturated fatty acid of 16-18 combines it with the 2nd place, In order to manufacture 1 and triglyceride which unsaturated fatty acid of omega3, omega6, and/or omega9 system combined with the 3rd place, if reaction temperature becomes high, in addition to a life of an enzyme becoming short, a danger that a higher unsaturated fatty acid will denaturalize is included.

[0010]Then, a result wholeheartedly studied in order that this invention persons might solve above-mentioned SUBJECT, To glyceride which saturated fatty acid of 16-18 has combined, a carbon number at

the 2nd place. Lipase which acts on 1 and an ester bond of the 3rd place specifically is made to act, At least one fatty acid of 1 and the 3rd place faces manufacturing triglyceride used as unsaturated fatty acid of omega3, omega6, and/or omega9 system by an ester exchange reaction, Fatty acid of the 2nd place of triglyceride is [a carbon number] once saturated fatty acid of 16-18, the melting point 1 and whose fatty acid of the 3rd place are medium chain fatty acid makes it go via it as an intermediate, using triglyceride 45 ** or less as a raw material -- it found out that target triglyceride could be manufactured and this invention was completed.

[0011]

[Embodiment of the Invention]According to this invention, in a carbon number, the saturated fatty acid of 16-18 combines with the 2nd place of triglyceride, either [at least] 1 or the 3rd place -- omega3, omega6, and/or omega -- the triglyceride which unsaturated fatty acid combined 9 system, a carbon number uses for the 2nd place as a substrate the triglyceride which the saturated fatty acid of 16-18 combined -- omega3, omega6, and/or omega -- it can manufacture under existence of unsaturated fatty acid or its ester 9 system by the ester exchange reaction by 1 and the lipase which acts on the 3rd place specifically.

[0012]Although a carbon number can mention tripalmitin (1, 2, and all of the 3rd place are pulmitic acid (16:0)), and a tristearin (1, 2, and all of the 3rd place are stearic acid (18:0)) to the 2nd place as triglyceride which the saturated fatty acid of 16-18 combined, for example, When the carbon number of the composition saturated fatty acid of triglyceride is 16 or more, this -- 1 and the 3rd place -- specific lipase, omega3, omega6, or omega -- when unsaturated fatty acid is made to react below 50 ** 9 system in the system of reaction which does not contain an organic solvent, the ester exchange reaction in 1 and the 3rd place hardly progresses, and triglyceride with the target structure is not obtained.

[0013]This originates in the character in which it hardly acts on the fats and oils of a solid state, although lipase acts on liquid fats and oils. Therefore, if the carbon number of the composition saturated fatty acid of triglyceride increases, the melting point needs to make reaction temperature high according to the part and this which become high. For example, when using tripalmitin, although it changes with reaction mixture presentations, a reaction must be performed at 50-70 **. For this reason, inactivation of an enzyme and the denaturation of the unsaturated fatty acid added for the ester interchange pose a problem.

[0014]So, when using triglyceride with these high melting points as a substrate raw material. Before exchanging for the unsaturated fatty acid aiming at 1 and fatty acid of the 3rd place by an ester interchange, The fatty acid combined with 1 of raw material triglyceride, and/or the 3rd place For example, with a carbon number of about eight to 12 medium chain fatty acid or oleic acid like caprylic acid, The ester interchange was carried out to fatty acid with the low melting point of linolic acid etc., and it was shown clearly that it is good to use the triglyceride which reduced the melting point at 45 ** or less as a raw material.

[0015]The higher unsaturated fatty acid once combined with the 1st place or the 3rd place in this method, After that Further 1, since it is hard to cause an ester interchange and the ester interchange of the medium chain fatty acid is preferentially carried out, even if it makes specific lipase act the 3rd place, in a carbon number, by repeating a reaction, the saturated fatty acid of 16-18 combines with the 2nd place of the purpose -- 1 and/or the 3rd place -- omega3, omega6, and/or omega -- it clarified that the yield of the triglyceride which unsaturated fatty acid combined 9 system could also be made to increase.

[0016]In order to clarify the feature of this invention, all the fatty acid combined with triglyceride was the same, and explained to the example the case where it was the saturated fatty acid of the carbon numbers

16-18, but. If not all the fatty acid that carries out an ester bond to triglyceride needs to be the same and the saturated fatty acid of the carbon numbers 16-18 has combined with the 2nd place of triglyceride, What kind of fatty acid of the carbon numbers 4-18 may combine with 1 and the 3rd place, or what kind of combination may be sufficient again, and using as a substrate the triglyceride which can react below 45 ** is included in the technical scope of this invention.

[0017]With the triglyceride which saturated fatty acid combined with the 2nd place. If the saturated fatty acid of the carbon numbers 16-18 has combined with the 2nd place, considering the purpose of this invention, either 1 and the 3rd place -- omega3, omega6, or omega -- unsaturated fatty acid having joined together 9 system, and, the position which has not combined unsaturated fatty acid when these substrates are used -- omega3, omega6, or omega -- unsaturated fatty acid can be introduced in an ester interchange 9 system, and the content of the unsaturated fatty acid of omega3 and omega6 which have been combined with 1 and the 3rd place, and/or omega9 system can be raised.

[0018]For example, the 2nd place as triglyceride which unsaturated fatty acid combined with either 1 and the 3rd place with saturated fatty acid, A KURIPUTEKODENIUMU (Crypthecodenium) group, the Thraustochytrium (Thraustochytrium) group, The fats and oils produced by cultivating the microorganism of the Schizochytrium (Schizochytrium) group, a Ur Kenya (Ulkenia) group, a Japonochytrium (Japonochytorium) group, or a HARIFO tris (Haliphthoros) group can be used.

[0019]1 and 2-dipalmitoyl 3-docosahexanolytriglyceride can be isolated from these, for example, this triglyceride -- a substrate -- 1 -- making specific lipase act the 3rd place -- omega3, omega6, or omega, if an ester interchange is carried out to unsaturated fatty acid or its fatty acid ester 9 system, As mentioned above, since the ester interchange of most docosahexaenoic acid is not carried out, the ester interchange only of the pulmitic acid of the 1st place is carried out. When arachidonic acid is used as unsaturated fatty acid, the triglyceride which docosahexaenoic acid combined with either 1 or the 3rd place, arachidonic acid combined with another side, and pulmitic acid combined with the 2nd place can be manufactured.

[0020]At least 1 of triglyceride and 3 can use specific lipase for this invention as a catalyst, Although not limited in particular, for example The Rhizopus (Rhizopus) group, A RIZOMU call (Rhizomucor) group, the Mucor (Mucor) group,Lipase, a swine pancreatic lipase, etc. which microorganisms, such as a penicillium (Penicillium) group, an Aspergillus (Aspergillus) group, the Humicola (Humicola) group, and a fusarium (Fusarium) group, produce are mentioned. A commercial thing can be used about this lipase.

[0021]For example, lipase of Rhizopus delemar (Rhizopusdelemar) (Tanabe Seiyaku Co., Ltd. make; TARIPAZE), Lipase of RIZOMU call MIIHAI (Rhizomucormiehei) (Novo Nordisk make; ribozyme IM), Lipase of Aspergillus-niger (Aspergillus niger) (the product made from Amano Pharmaceuticals; lipase A), Lipase of the Humicola RANGI norther (Humicolalanuginosa) (Novo Nordisk make; RIPORAZE), Lipase (the product made from Amano Pharmaceuticals; lipase M) of Mucor Java NIKASU (Mucorjavanicus), lipase of fusarium hetero SUPORAMU (Fusariumheterosporum), etc. are mentioned. the using form of these lipase may use the lipase which it could come out of as it was, and could be used, and was fixed in cerite, ion-exchange resin, a ceramic carrier, etc.

[0022]The moisture content applied to this system of reaction is very important, and when water is not included at all, an ester interchange does not advance, When there are many moisture contents, hydrolysis takes place, the recovery rate of triglyceride falls, or spontaneous acyl group transfer happens in the generated partial glycerides, and the saturated fatty acid of the 2nd place transfers to the 1st place or the

3rd place. Therefore, when immobilized enzyme without absorbed water is used, before performing a main reaction, it is effective if the substrate which activates an enzyme first using the substrate which added water, and has not added water in a main reaction is used. In order to pretreat an enzyme using the substrate which contains 0-1 of the applied amount of enzymes, and 000% (% of the weight) of water in order to be activated by a batch reaction and to be activated with a column method, it is good to pour the substrate of water saturation continuously.

[0023]For example, when activated by a batch reaction using lipase (Tanabe Seiyaku Co., Ltd. make; TARIPAZE) of Rhizopus delemar (Rhizopusdelemar) fixed in cerite or a ceramic carrier, a moisture content is 10 to 200% of the applied amount of enzymes (% of the weight). However, a moisture content required for activation of an ester exchange reaction is greatly influenced by the kind of enzyme to be used, For example, if it is lipase (Novo Nordisk make; ribozyme IM) of RIZOMU call MIIHAI (Rhizomucormiehei), moisture is hardly needed but superfluous water must be removed rather. Removal of superfluous water is good to choose as a substrate the triglyceride which does not block a main reaction, and for immobilized enzyme to hydrolyze this.

[0024]What is necessary is for a reaction condition just to determine suitably the amount of the lipase used in a batch reaction, Lipase of Rhizopus delemar (Rhizopusdelemar) fixed, for example in cerite or a ceramic carrier although not restricted in particular, Or when lipase of RIZOMU call MIIHAI (Rhizomucormiehei) is used, 1 to 30% of cocktails (% of the weight) are optimum dose.

[0025]The ester exchange reaction in a batch reaction is performed by the following methods. namely, the triglyceride which the saturated fatty acid of 16-18 combined with the 2nd place in the carbon number -- omega3, omega6, or omega -- unsaturated fatty acid or its fatty acid ester is added 9 system. As fatty acid ester, methyl ester, ethyl ester, propyl ester, butylester, etc. can be used, for example. As for the triglyceride / fatty acid or the triglyceride / fatty-acid-ester ratio used as a raw material, 1:0. 5-20 are optimum dose. Suitable quantity for this substrate (usual [5], 000-50, and 000 U/g; in the lipase 1U here.) What is necessary is just to perform 45 ** or less of ester exchange reactions near 30 ** preferably for 2 to 100 hours, at least 1 of being the amount of enzymes which separates fatty acid of 1micromol in 1 minute which activated or dried, and 3 adding specific lipase, and stirring or shaking them using olive oil as a substrate.

[0026]Repeated use of the above-mentioned immobilized enzyme can be carried out. That is, a reaction is continuable by leaving only after-reaction immobilized enzyme in a reactor, and exchanging reaction mixture for the newly prepared substrate. The ester exchange reaction by a column method is good to pour a substrate continuously by per [enzyme 1g], and 0.05 - 20 ml/hr. The target triglyceride content can be raised by repeating an ester exchange reaction and performing it. namely, omega3, omega6, or omega9 system -- the bottom of existence of unsaturated fatty acid or its fatty acid ester -- 1 of triglyceride -- making specific lipase act the 3rd place -- fatty acid of 1 and the 3rd place -- omega3, omega6, and/or omega -- the reaction mixture by which the ester interchange was carried out to unsaturated fatty acid 9 system is obtained.

[0027]next -- refining triglyceride by the method of mentioning later from this reaction solution, and using this refining triglyceride as a raw material -- again -- omega3, omega6, or omega -- unsaturated fatty acid or its fatty acid ester performs an ester exchange reaction 9 system. This repetition esterification reaction can raise the target triglyceride content by leaps and bounds, and 2 to 5 times of repeat frequency are preferred.

[0028]In the ester exchange reaction using conventional fixed lipase, the acyl group transfer of the fatty acid combined with the 2nd place of the partial glycerides generated by the hydrolysis reaction which occurs as a side reaction was induced. However, in this invention, the hydrolysis reaction could be suppressed nearly thoroughly, and the generated amount of partial glycerides is a grade 1%, and was able to solve the conventional problem. If the moisture content contained in the substrate is thousands of ppm or less, the hydrolysis which takes place as a side reaction can be disregarded, and it has the feature that it is not necessary to carry out close control of the moisture content contained in a substrate.

[0029]It receives that enzyme activity fell by several use at a reaction in the organic solvent using conventional immobilized enzyme, or a not less than 50 ** reaction, It is also possible for inactivation of an enzyme not to take place, in order to react below 45 ** according to the system of reaction which does not use an organic solvent in this invention, but to use an enzyme continuously 100 days or more at a column reaction tens times or more by a batch reaction.

[0030]By this invention, since the substrate is simple, the triglyceride obtained by a reaction comprises several sorts of molecular species. Then, target triglyceride can be easily isolated with conventional methods, such as liquid chromatography, molecular distillation, flowing-down membrane distillation, and superfractionation, or the combination of those. Triglyceride after the reaction manufactured by this invention, It is the triglyceride which unsaturated fatty acid combined with the 1st place and/or the 3rd place, It exists as a mixture with fatty acid or this fatty acid ester combined with 1 of triglyceride of this triglyceride, an unreacted raw material and unreacted unsaturated fatty acid or fatty acid ester, and the raw material that the ester interchange was carried out and was produced, and/or the 3rd place.

[0031]Then, refining of the triglyceride which unsaturated fatty acid combined with the 1st place of the purpose, and/or the 3rd place, and the saturated fatty acid of 16-18 combined with the 2nd place in the carbon number, It can carry out by removing above-mentioned fatty acid and unreacted unsaturated fatty acid by which the ester interchange was carried out by combining alkali deoxidation, steam distillation, molecular distillation, flowing-down membrane distillation, vacuum superfractionation, column chromatography, solvent extraction or membrane separation, or these.

[0032]the fatty acid which constitutes 1 of the triglyceride obtained by this invention, and the 3rd place -- omega3, omega6, and/or omega -- it consists of unsaturated fatty acid 9 system. concrete -- omega3 system -- as unsaturated fatty acid -- 9, 12, and 15-octadecatrienoic acid [(alpha- linolenic acid)] -- [18:3, omega3]. 6,9, 12, 15 - Octadeca tetraenoic acid (steer RIDON acid) [18:4, omega3], 11, 14, and 17-eicosatrienoic acid (******- alpha-linoleic acid) -- [20:3, omega3]. 8, 11, 14, 17-eicosatetraenoic acid [20:4, omega3], 5, 8, 11, 14, 17-eicosapentaenoic acid [20:5, omega3], 7, 10, 13, 16, 19-docosapentaenoic acid [22:5, omega3], 4, 7, 10, 13, 16, and 19- docosahexaenoic acid [22:6, omega3] can be mentioned.

[0033]omega6 system -- as unsaturated fatty acid -- 9 and 12-octadecadienoic acid [(linolic acid)] -- [18:2, omega6]. 6, 9, 12-octadecatrienoic acid (gamma- linolenic acid) [18:3, omega6], 8, 11, 14-eicosatrienoic acid (******- gamma-linolenic acid) [20:3, omega6], 5, 8, 11, 14-eicosatetraenoic acid (arachidonic acid) [20:4, omega6], 7, 10, 13, 16-docosatetraenoic acid [22:4, omega6], 4, 7, 10, 13, 16, and -docosapentaenoic acid [22:3, omega6] can be mentioned. [20:3, omega9] 11- eicosatrienoic-acid (mead acid) omega9 system -- as unsaturated fatty acid -- 6, 9- octadecadienoic acid [18:2, omega9], 8, 11-eicosadienoic acid [20:2, omega9], 5, and 8 -- it can mention. An acyl group may be hydroxylation, epoxidation, or an acyl group by which hydroxy epoxidation was carried out. The fatty acid which constitutes

the 2nd place of new triglyceride of this invention consists of fatty acid of the carbon numbers 16-18. For example, pulmitic acid (16:0) and stearic acid (18:0) can be mentioned.

[0034]

[Example]Next, an example explains this invention still more concretely. In this example, the following cable addresses show fatty acid and triglyceride for convenience. First, the following are used for the single-character cable address showing fatty acid. 8: Caprylic acid, P:pulmitic acid, A:arachidonic acid, M:mead acid, D : docosahexaenoic acid. Next, it writes by three characters with the single-character cable address showing the fatty acid which has combined triglyceride with the 1st place, the single-character cable address showing the fatty acid combined with the 2nd place, and the single-character cable address showing the fatty acid combined with the 3rd place. Therefore, the structure of triglyceride is written like the following example. Example: 8P8 (triglyceride which caprylic acid combined with caprylic acid at the 1st place, and combined with the 2nd place at pulmitic acid and the 3rd place)

[0035]1:2 (wt/wt) mixture of example 1. tripalmitin (PPP) and caprylic acid is used as a substrate raw material, The reaction mixture which turns into 10.5 g of substrate mixture from 1.2g of fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) is put into a vial bottle with a screw cap, It incubated shaking at 50 ** for 48 hours (a part for 140 times/). After the reaction, it left only immobilized enzyme, reaction mixture was exchanged for new substrate mixture, and it reacted under the same conditions. The reaction was performed 4 times, carrying out repeated use of the immobilized enzyme, and each reaction mixture was collected.

[0036]A 70-ml 0.5N KOH solution (20% ethanol solution) was added to each reaction mixture (10.5g), the evaporator removed the solvent after extracting a glyceride fraction by 100 ml of hexane, and glyceride was collected. As a result of investigating a glyceride presentation by an IYATO loss can (made by YATORON), 8% of diglyceride was contained in the 1st glyceride, but the partial-glycerides content in glyceride of the 2nd henceforth was 1% or less. The fatty acid composition (mol %) of the 2-4th glyceride fractions was 45.1% of caprylic acid, and 54.9% of pulmitic acid.

[0037]In order to raise the replacement factor of caprylic acid, the 2-4th glyceride fractions were used as the raw material, and the ester interchange was carried out again. The glyceride 3.5g and the caprylic acid 7g which were prepared were added to ribozyme IM60 (1.2g) used for the above-mentioned reaction, and it reacted, shaking at 30 ** for 48 hours (the 5th time). After the reaction, reaction mixture was exchanged for a new substrate and it reacted under the same conditions (the 6th time). Hexane extraction recovered the glyceride fraction from the 5 or 6th reaction mixture (a total of four .8 g). The fatty acid composition (mol %) of the obtained glyceride was 64.2% of caprylic acid, and 35.8% of pulmitic acid. The result which the partial glycerides contained in this glyceride are the following 1%, and was analyzed with the ODS column (Wakosil-II 3C18, 4.6x150 mm, and 2) by using acetone/acetonitrile (1:1, vol/vol) as an elution solvent, The purity of 8P8 was 93%.

[0038]8P8 (3.5g) and 7 g of arachidonic acid (90% of purity) which were obtained were used as the raw material, the ester exchange reaction was performed at 30 ** ribozyme IM60 used for the above-mentioned reaction for 48 hours (the 7th time), hexane extraction of the reaction mixture after a reaction was carried out under alkali conditions, and the glyceride fraction (4.8g) was obtained. When the fatty acid composition of glyceride was analyzed, caprylic acid, pulmitic acid, gamma- linolenic acid, and arachidonic acid were 38.5, 23.1, 2.4, and 34.0-mol %, respectively. The result of having carried out fractionation of this glyceride with

the high performance chromatography using an ODS column (SH-345-5, product made from 20 x 500mm YMC) by using acetone/acetonitrile (1:1, vol/vol) as an elution solvent, 8PA and 0.72 or 0.44g of APA were obtained, respectively.
[0039]It reacted on a scale of 100 times of the method indicated in the example 2. example 1, 8P8 was prepared, and it was used as a raw material. Lipase (Tanabe Seiyaku Co., Ltd. make; TARIPAZE) of Rhizopus delemar (Rhizopusdelemar) was fixed in ceramic carrier SM-10 (made by NGK Insulators, Ltd.) according to J. Ferment. Bioeng., 81, and 299-303 (1996). Soybean oil of water saturation after filling up a column with the immobilized enzyme 10g (31, 000 U/g): 100-ml sink immobilized enzyme was activated for caprylic acid 1:2 (wt/wt) mixed liquor by 30 ** and rate-of-flow 3 ml/hr.
[0040]Subsequently, after pouring 50 ml of soybean oil which is not adding water and removing superfluous water, the ester exchange reaction was performed, passing 1:4 (wt/wt) mixture of 8P8 and arachidonic acid ethyl ester (90% of purity) on the same conditions. After distilling 100 g of reaction mixture under the high vacuum and collecting glyceride fractions as residue, according to Example 1, hexane extraction was carried out under alkali conditions. The evaporator removed the solvent and 35.7 g hexane extractable material was obtained. It was 91:9 when the composition ratio of triglyceride and fatty acid ester which are contained in this hexane extractable material was analyzed by the IYATO loss can. As a result of analyzing fatty acid composition, they are caprylic acid, pulmitic acid, gamma- linolenic acid, and ******-. gamma- linolenic acid and arachidonic acid were 24.4, 34.5, 1.5, 2.6, and 37.0-mol %, respectively.
[0041]In order to remove the superfluous water contained in the fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) used in example 3. example 1, This immobilized enzyme 12g and SUNTGA - The cocktail which consists of 25 (made by Suntory) 60g is put into a 100-ml vial bottle with a screw cap, and it was made to react, shaking at 30 ** for 48 hours (the 1st time). It left only immobilized enzyme to the reactor, and after adding 8P8 (12g) and 48 g of mead acid ethyl ester (90% of purity) which were created in Example 2 and carrying out a nitrogen purge enough, the ester exchange reaction was performed, shaking at 30 ** for 72 hours (the 2 or 3rd time).
[0042]After the reaction, 100 g of them including the 2nd time and the 3rd cocktail was high vacuum distilled like Example 2, and glyceride fractions were collected as residue. Subsequently, after carrying out hexane extraction under alkali conditions according to Example 1, the evaporator removed hexane and a 24.1-g glyceride fraction was obtained. It was 92:8 when the composition ratio of triglyceride and fatty acid ester which are contained in this was analyzed by the IYATO loss can. High performance chromatography was performed according to Example 1, and MPM was 12.0% about a fixed quantity of fatty acid ester and each triglyceride ingredients from the peak area of a differential refractometer at the bottom and the time. Caprylic acid, pulmitic acid, and the mead acid of the fatty acid composition of this fraction were 31.2, 35.7, and 33.1-mol %, respectively.
[0043]In order to raise a transesterification rate, the ester interchange of the obtained ester interchange triglyceride was again carried out by mead acid ethyl ester. It reacted, having added the ester interchange triglyceride 12g and 48 g of mead acid ethyl ester to the above-mentioned immobilized enzyme, and shaking at 30 ** for 72 hours (the 4th time). It distilled after the reaction by the method which mentioned above 55 g of reaction mixture, and the glyceride fraction of 12.3 g was obtained. Caprylic acid, pulmitic acid, and the mead acid of the fatty acid composition of this fraction were 5.2, 38.6, and 56.1-mol %, respectively.
[0044]In order to remove the superfluous water contained in the fixed RIZOMU call MIIHAI

(Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) used in example 4. example 1, This immobilized enzyme 2g and SUNTGA - The cocktail which consists of 25 (made by Suntory) 10g is put into a 20-ml vial bottle with a screw cap, and it was made to react, shaking at 30 ** for 48 hours (the 1st time). 8P8 (12g) and SUNTGA which left only immobilized enzyme to the reactor and were created in Example 2 - After adding 8 g of fatty acid mixture produced by hydrolyzing 25 and carrying out a nitrogen purge enough, the ester exchange reaction was performed, shaking at 30 ** for 48 hours (the 2-5th time). The glyceride which carried out hexane extraction from the 2-5th cocktails was set after the reaction, and it was considered as the substrate of the ester exchange reaction for the second time.

[0045]They are the ester interchange triglyceride 2g and SUNTGA to the reactor containing the above-mentioned immobilized enzyme. - 10 g of fatty acid mixture of 25 origin is added, and it was made to react, shaking at 30 ** for 48 hours (the 6 or 7th time). The glyceride fraction was extracted from the 6 or 7th cocktail, and it was considered as the substrate of the ester exchange reaction of a third-time degree, and reacted similarly (the 8th time). Gas chromatography analyzed each fatty acid composition, the fatty acid composition which constitutes the triglyceride obtained by repeating an ester exchange reaction 3 times, and triglyceride, of the 1 or 3rd place and the 2nd place. This result is shown in Table 1.

[0046]

[Table 1]

表１（単位：モル％）

脂肪酸の種類	新規構造脂質		
	全体	1，3位	2位
8：0	9	9	2
16：0	34	6	96
18：1（n-9）	11	16	0
18：2（n-6）	15	22	1
18：3（n-6）	2	3	1
20：3（n-6）	1	3	0
20：4（n-6）	15	23	0

[0047]8P8 and immobilized enzyme which were created in comparative example 1. example 2 were used as a raw material and a catalyst, respectively. The immobilized enzyme 2g, the soybean oil 4g, the caprylic acid 8g, and the water 0.5g were put into a 20-ml vial bottle, and immobilized enzyme was activated by incubating shaking at 30 ** for 24 hours. It left the activated enzyme in the reactor and the substrate, the arachidonic acid /8P8 which does not contain water in this (4:1, wt/wt), or arachidonic acid/PPP (4:1, wt/wt) was added, and it carried out, having shaken the former reaction at 30 ** and shaking the latter reaction at 50 **. The reaction compared the stability of immobilized enzyme repeatedly, exchanging reaction mixture for the substrate which will seemingly be new every 24 hours.

[0048]When PPP is used for a substrate and a reaction is repeated at 50 **, after using immobilized enzyme 7 times, the uptake quantity of arachidonic acid fell to 10% or less of the first uptake quantity (the uptake quantity of the 1st time and the 7th arachidonic acid is 47% and 3%, respectively). When 8P8 was used for a substrate and a reaction was repeated at 30 ** on the other hand, even if it used immobilized enzyme 50 times, the uptake quantity of arachidonic acid hardly changed (the uptake quantity of the 1st time and the 50th arachidonic acid is 41% and 38%, respectively).

[Translation done.]

TECHNICAL FIELD

[Field of the Invention]This invention relates to the manufacturing method of new triglyceride, and relates to the manufacturing method of the triglyceride which has the saturated fatty acid of the carbon numbers 16-18 especially in the 2nd place of triglyceride, and has the unsaturated fatty acid of omega3, omega6, and/or omega9 system in either [at least] 1 or the 3rd place.

[Translation done.]

PRIOR ART

[Description of the Prior Art]The great portion of lipid which we are taking in is neutral fat, and it is a mixture of the triglyceride in which 1 of triglyceride, 2, and various fatty acid carried out the ester bond to the 3rd place. And it is pointed out by the difference in the connecting position of fatty acid that the physiology activity differs, and the lipid (structure lipid) which combined specific fatty acid with the position it was decided that triglyceride would be attracts attention especially these days.

[0003]for example, JP,4-12920,B **** -- good triglyceride of the digestion nature which fatty acid of the carbon numbers 8-14 combined with the 2nd place of triglyceride, and fatty acid whose carbon number is 18 or more combined with 1 and the 3rd place is indicated. 2 - JP,5-87497,B since monoglyceride is considered to be a gestalt which tended to be absorbed by people's living body ****. omega3 which has a physiological function in the 2nd place, or omega -- the triglyceride which combined the higher unsaturated fatty acid 6 system, and combined the saturated fatty acid easily hydrolyzed into 1 and the 3rd place with the enzyme of an alimentary canal is indicated.

[0004]On the other hand, if their eyes are turned to the physiological function of fatty acid, arachidonic acid and docosahexaenoic acid attract attention in recent years. These fatty acid is contained in mother's milk. A report ("Advances in Polyunsaturated Fatty Acid Research", Elsevier Science Publishers, 1993, pp.261-264) that it is useful for a suckling's growth, There is a report (Proc. Natl. Acad. Sci. USA, 90, 1073-1077 (1993), Lancet, 344, and 1319-1322 (1994)) of being important for embryonic height or cerebral growth.

[0005]And a recommended intake is released from some public institutions (premature baby: docosahexaenoic acid 20 mg/kg weight / [the arachidonic acid 60, the docosahexaenoic acid 40; normal-child:arachidonic acid 20, and] day (WHO-FAO (1994)).). In the several countries in Europe, the modified milk for premature babies which blended as triglyceride the arachidonic acid which already combined with docosahexaenoic acid and carried out fermentation production is marketed. However, it is not taken into consideration about the connecting position of the arachidonic acid of triglyceride, and/or docosahexaenoic acid applied to modified milk.

[0006]The triglyceride structure in people's mother's milk has a high rate which pulmitic acid (16:0) combines with the 2nd place of triglyceride, 1. To the 3rd place, and a higher unsaturated fatty acid. . Or it is thought that the rate which medium chain fatty acid combines is high. (Christie, W.W. (1986) The Positional Distribution of Fatty Acids in Triglycerids. Analysis of Oils and Fats in (Hamilton,) R.J., and Russell, J.B., eds.pp. 313-339, Elsevier Applied Science, and London.

[0007]On the other hand, the structure of the arachidonic acid content triglyceride produced by the fermenting method added to modified milk in order to bring the above-mentioned fatty acid composition close to the presentation of mother's milk, Saturated fatty acid including pulmitic acid combines with 1 and the 3rd place, The rate combined with the 2nd place unsaturated fatty acid highly (J. J. Myher, A. Kuksis, K. Geher, P.W. Park, and D.A Diersen-Schade, Lipids 31, and 207-215 (1996)), It differed from what is considered to be people's mother's milk type clearly. Therefore, development of the structure lipid by which structure is checked clearly which the carbon number combined with the 2nd place (the structure lipid considered to be people's mother's milk type triglyceride structure, i.e., triglyceride), and a higher unsaturated fatty acid or medium chain fatty acid combined with the saturated fatty acid of 16-18, 1, and the 3rd place is desired strongly.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention]Therefore, the structure lipid by which this invention is considered to be a Homo sapiens mother's milk type triglyceride structure, That is, the 2nd place of triglyceride is [a carbon number] the saturated fatty acid of 16-18, the unsaturated fatty acid combined with 1 and the 3rd place -- at least -- one -- omega3, omega6, or omega -- new triglyceride which is unsaturated fatty acid 9 system. or -- the 2nd place of triglyceride is [a carbon number] the saturated fatty acid of 16-18, and either 1 or the 3rd place is [a carbon number] the saturated fatty acid of 4-18 -- another side -- omega3, omega6, or omega -- it is going to provide the manufacturing method of new triglyceride which is unsaturated fatty acid 9 system.

[Translation done.]

MEANS

[Means for Solving the Problem]A method of manufacturing triglyceride which fatty acid of the carbon numbers 8-14 combined with the 2nd place of triglyceride by 1 and an ester exchange reaction using specific lipase the 3rd place, and fatty acid whose carbon number is 18 or more combined with 1 and the 3rd place is above-mentioned JP,4-12920,B. It is indicated. However, in order for fatty acid of the 2nd place to use as a raw material triglyceride in which a carbon number consists of saturated fatty acid of the carbon numbers 16-18 which increased further and at least for 1 and 3 to perform an ester exchange reaction with unsaturated fatty acid of omega3, omega6, or omega9 system using specific lipase, reaction temperature must be not less than 50 **. This reaction is a reaction which used immobilized enzyme, and, in a carbon number, saturated fatty acid of 16-18 combines it with the 2nd place, In order to manufacture 1 and triglyceride which unsaturated fatty acid of omega3, omega6, and/or omega9 system combined with the 3rd place, if reaction temperature becomes high, in addition to a life of an enzyme becoming short, a danger that a higher unsaturated fatty acid will denaturalize is included.

[0010]Then, a result wholeheartedly studied in order that this invention persons might solve above-mentioned SUBJECT, To glyceride which saturated fatty acid of 16-18 has combined, a carbon number at the 2nd place. Lipase which acts on 1 and an ester bond of the 3rd place specifically is made to act, At least one fatty acid of 1 and the 3rd place faces manufacturing triglyceride used as unsaturated fatty acid of omega3, omega6, and/or omega9 system by an ester exchange reaction, Fatty acid of the 2nd place of triglyceride is [a carbon number] once saturated fatty acid of 16-18, the melting point 1 and whose fatty acid of the 3rd place are medium chain fatty acid makes it go via it as an intermediate, using triglyceride 45 ** or less as a raw material -- it found out that target triglyceride could be manufactured and this invention was completed.

[0011]

[Embodiment of the Invention]According to this invention, in a carbon number, the saturated fatty acid of 16-18 combines with the 2nd place of triglyceride, either [at least] 1 or the 3rd place -- omega3, omega6, and/or omega -- the triglyceride which unsaturated fatty acid combined 9 system, a carbon number uses for the 2nd place as a substrate the triglyceride which the saturated fatty acid of 16-18 combined -- omega3, omega6, and/or omega -- it can manufacture under existence of unsaturated fatty acid or its ester 9 system by the ester exchange reaction by 1 and the lipase which acts on the 3rd place specifically.

[0012]Although a carbon number can mention tripalmitin (1, 2, and all of the 3rd place are pulmitic acid (16:0)), and a tristearin (1, 2, and all of the 3rd place are stearic acid (18:0)) to the 2nd place as triglyceride

which the saturated fatty acid of 16-18 combined, for example, When the carbon number of the composition saturated fatty acid of triglyceride is 16 or more, this -- 1 and the 3rd place -- specific lipase, omega3, omega6, or omega -- when unsaturated fatty acid is made to react below 50 ** 9 system in the system of reaction which does not contain an organic solvent, the ester exchange reaction in 1 and the 3rd place hardly progresses, and triglyceride with the target structure is not obtained.

[0013]This originates in the character in which it hardly acts on the fats and oils of a solid state, although lipase acts on liquid fats and oils. Therefore, if the carbon number of the composition saturated fatty acid of triglyceride increases, the melting point needs to make reaction temperature high according to the part and this which become high. For example, when using tripalmitin, although it changes with reaction mixture presentations, a reaction must be performed at 50-70 **. For this reason, inactivation of an enzyme and the denaturation of the unsaturated fatty acid added for the ester interchange pose a problem.

[0014]So, when using triglyceride with these high melting points as a substrate raw material. Before exchanging for the unsaturated fatty acid aiming at 1 and fatty acid of the 3rd place by an ester interchange, The fatty acid combined with 1 of raw material triglyceride, and/or the 3rd place For example, with a carbon number of about eight to 12 medium chain fatty acid or oleic acid like caprylic acid, The ester interchange was carried out to fatty acid with the low melting point of linolic acid etc., and it was shown clearly that it is good to use the triglyceride which reduced the melting point at 45 ** or less as a raw material.

[0015]The higher unsaturated fatty acid once combined with the 1st place or the 3rd place in this method, After that Further 1, since it is hard to cause an ester interchange and the ester interchange of the medium chain fatty acid is preferentially carried out, even if it makes specific lipase act the 3rd place, in a carbon number, by repeating a reaction, the saturated fatty acid of 16-18 combines with the 2nd place of the purpose -- 1 and/or the 3rd place -- omega3, omega6, and/or omega -- it clarified that the yield of the triglyceride which unsaturated fatty acid combined 9 system could also be made to increase.

[0016]In order to clarify the feature of this invention, all the fatty acid combined with triglyceride was the same, and explained to the example the case where it was the saturated fatty acid of the carbon numbers 16-18, but. If not all the fatty acid that carries out an ester bond to triglyceride needs to be the same and the saturated fatty acid of the carbon numbers 16-18 has combined with the 2nd place of triglyceride, What kind of fatty acid of the carbon numbers 4-18 may combine with 1 and the 3rd place, or what kind of combination may be sufficient again, and using as a substrate the triglyceride which can react below 45 ** is included in the technical scope of this invention.

[0017]With the triglyceride which saturated fatty acid combined with the 2nd place. If the saturated fatty acid of the carbon numbers 16-18 has combined with the 2nd place, considering the purpose of this invention, either 1 and the 3rd place -- omega3, omega6, or omega -- unsaturated fatty acid having joined together 9 system, and, the position which has not combined unsaturated fatty acid when these substrates are used -- omega3, omega6, or omega -- unsaturated fatty acid can be introduced in an ester interchange 9 system, and the content of the unsaturated fatty acid of omega3 and omega6 which have been combined with 1 and the 3rd place, and/or omega9 system can be raised.

[0018]For example, the 2nd place as triglyceride which unsaturated fatty acid combined with either 1 and the 3rd place with saturated fatty acid, A KURIPUTEKODENIUMU (Crypthecodenium) group, the Thraustochytrium (Thraustochytrium) group, The fats and oils produced by cultivating the microorganism of the Schizochytrium (Schizochytrium) group, a Ur Kenya (Ulkenia) group, a Japonochytrium

(Japonochytorium) group, or a HARIFO tris (Haliphthoros) group can be used.
[0019]1 and 2-dipalmitoyl 3-docosahexanolytriglyceride can be isolated from these, for example, this triglyceride -- a substrate -- 1 -- making specific lipase act the 3rd place -- omega3, omega6, or omega, if an ester interchange is carried out to unsaturated fatty acid or its fatty acid ester 9 system, As mentioned above, since the ester interchange of most docosahexaenoic acid is not carried out, the ester interchange only of the pulmitic acid of the 1st place is carried out. When arachidonic acid is used as unsaturated fatty acid, the triglyceride which docosahexaenoic acid combined with either 1 or the 3rd place, arachidonic acid combined with another side, and pulmitic acid combined with the 2nd place can be manufactured.
[0020]At least 1 of triglyceride and 3 can use specific lipase for this invention as a catalyst, Although not limited in particular, for example The Rhizopus (Rhizopus) group, A RIZOMU call (Rhizomucor) group, the Mucor (Mucor) group,Lipase, a swine pancreatic lipase, etc. which microorganisms, such as a penicillium (Penicillium) group, an Aspergillus (Aspergillus) group, the Humicola (Humicola) group, and a fusarium (Fusarium) group, produce are mentioned. A commercial thing can be used about this lipase.
[0021]For example, lipase of Rhizopus delemar (Rhizopusdelemar) (Tanabe Seiyaku Co., Ltd. make; TARIPAZE), Lipase of RIZOMU call MIIHAI (Rhizomucormiehei) (Novo Nordisk make; ribozyme IM), Lipase of Aspergillus-niger (Aspergillus niger) (the product made from Amano Pharmaceuticals; lipase A), Lipase of the Humicola RANGI norther (Humicolalanuginosa) (Novo Nordisk make; RIPORAZE), Lipase (the product made from Amano Pharmaceuticals; lipase M) of Mucor Java NIKASU (Mucorjavanicus), lipase of fusarium hetero SUPORAMU (Fusariumheterosporum), etc. are mentioned. the using form of these lipase may use the lipase which it could come out of as it was, and could be used, and was fixed in cerite, ion-exchange resin, a ceramic carrier, etc.
[0022]The moisture content applied to this system of reaction is very important, and when water is not included at all, an ester interchange does not advance, When there are many moisture contents, hydrolysis takes place, the recovery rate of triglyceride falls, or spontaneous acyl group transfer happens in the generated partial glycerides, and the saturated fatty acid of the 2nd place transfers to the 1st place or the 3rd place. Therefore, when immobilized enzyme without absorbed water is used, before performing a main reaction, it is effective if the substrate which activates an enzyme first using the substrate which added water, and has not added water in a main reaction is used. In order to pretreat an enzyme using the substrate which contains 0-1 of the applied amount of enzymes, and 000% (% of the weight) of water in order to be activated by a batch reaction and to be activated with a column method, it is good to pour the substrate of water saturation continuously.
[0023]For example, when activated by a batch reaction using lipase (Tanabe Seiyaku Co., Ltd. make; TARIPAZE) of Rhizopus delemar (Rhizopusdelemar) fixed in cerite or a ceramic carrier, a moisture content is 10 to 200% of the applied amount of enzymes (% of the weight). However, a moisture content required for activation of an ester exchange reaction is greatly influenced by the kind of enzyme to be used, For example, if it is lipase (Novo Nordisk make; ribozyme IM) of RIZOMU call MIIHAI (Rhizomucormiehei), moisture is hardly needed but superfluous water must be removed rather. Removal of superfluous water is good to choose as a substrate the triglyceride which does not block a main reaction, and for immobilized enzyme to hydrolyze this.
[0024]What is necessary is for a reaction condition just to determine suitably the amount of the lipase used in a batch reaction, Lipase of Rhizopus delemar (Rhizopusdelemar) fixed, for example in cerite or a ceramic

carrier although not restricted in particular, Or when lipase of RIZOMU call MIIHAI (Rhizomucormiehei) is used, 1 to 30% of cocktails (% of the weight) are optimum dose.
[0025]The ester exchange reaction in a batch reaction is performed by the following methods. namely, the triglyceride which the saturated fatty acid of 16-18 combined with the 2nd place in the carbon number -- omega3, omega6, or omega -- unsaturated fatty acid or its fatty acid ester is added 9 system. As fatty acid ester, methyl ester, ethyl ester, propyl ester, butylester, etc. can be used, for example. As for the triglyceride / fatty acid or the triglyceride / fatty-acid-ester ratio used as a raw material, 1:0. 5-20 are optimum dose. Suitable quantity for this substrate (usual [5], 000-50, and 000 U/g; in the lipase 1U here.) What is necessary is just to perform 45 ** or less of ester exchange reactions near 30 ** preferably for 2 to 100 hours, at least 1 of being the amount of enzymes which separates fatty acid of 1micromol in 1 minute which activated or dried, and 3 adding specific lipase, and stirring or shaking them using olive oil as a substrate.
[0026]Repeated use of the above-mentioned immobilized enzyme can be carried out. That is, a reaction is continuable by leaving only after-reaction immobilized enzyme in a reactor, and exchanging reaction mixture for the newly prepared substrate. The ester exchange reaction by a column method is good to pour a substrate continuously by per [enzyme 1g], and 0.05 - 20 ml/hr. The target triglyceride content can be raised by repeating an ester exchange reaction and performing it. namely, omega3, omega6, or omega9 system -- the bottom of existence of unsaturated fatty acid or its fatty acid ester -- 1 of triglyceride -- making specific lipase act the 3rd place -- fatty acid of 1 and the 3rd place -- omega3, omega6, and/or omega -- the reaction mixture by which the ester interchange was carried out to unsaturated fatty acid 9 system is obtained.
[0027]next -- refining triglyceride by the method of mentioning later from this reaction solution, and using this refining triglyceride as a raw material -- again -- omega3, omega6, or omega -- unsaturated fatty acid or its fatty acid ester performs an ester exchange reaction 9 system. This repetition esterification reaction can raise the target triglyceride content by leaps and bounds, and 2 to 5 times of repeat frequency are preferred.

[0028]In the ester exchange reaction using conventional fixed lipase, the acyl group transfer of the fatty acid combined with the 2nd place of the partial glycerides generated by the hydrolysis reaction which occurs as a side reaction was induced. However, in this invention, the hydrolysis reaction could be suppressed nearly thoroughly, and the generated amount of partial glycerides is a grade 1%, and was able to solve the conventional problem. If the moisture content contained in the substrate is thousands of ppm or less, the hydrolysis which takes place as a side reaction can be disregarded, and it has the feature that it is not necessary to carry out close control of the moisture content contained in a substrate.
[0029]It receives that enzyme activity fell by several use at a reaction in the organic solvent using conventional immobilized enzyme, or a not less than 50 ** reaction, It is also possible for inactivation of an enzyme not to take place, in order to react below 45 ** according to the system of reaction which does not use an organic solvent in this invention, but to use an enzyme continuously 100 days or more at a column reaction tens times or more by a batch reaction.
[0030]By this invention, since the substrate is simple, the triglyceride obtained by a reaction comprises several sorts of molecular species. Then, target triglyceride can be easily isolated with conventional methods, such as liquid chromatography, molecular distillation, flowing-down membrane distillation, and superfractionation, or the combination of those. Triglyceride after the reaction manufactured by this

invention, It is the triglyceride which unsaturated fatty acid combined with the 1st place and/or the 3rd place, It exists as a mixture with fatty acid or this fatty acid ester combined with 1 of triglyceride of this triglyceride, an unreacted raw material and unreacted unsaturated fatty acid or fatty acid ester, and the raw material that the ester interchange was carried out and was produced, and/or the 3rd place.

[0031]Then, refining of the triglyceride which unsaturated fatty acid combined with the 1st place of the purpose, and/or the 3rd place, and the saturated fatty acid of 16-18 combined with the 2nd place in the carbon number, It can carry out by removing above-mentioned fatty acid and unreacted unsaturated fatty acid by which the ester interchange was carried out by combining alkali deoxidation, steam distillation, molecular distillation, flowing-down membrane distillation, vacuum superfractionation, column chromatography, solvent extraction or membrane separation, or these.

[0032]the fatty acid which constitutes 1 of the triglyceride obtained by this invention, and the 3rd place -- omega3, omega6, and/or omega -- it consists of unsaturated fatty acid 9 system. concrete -- omega3 system -- as unsaturated fatty acid -- 9, 12, and 15-octadecatrienoic acid [(alpha- linolenic acid)] -- [18:3, omega3]. 6,9, 12, 15 - Octadeca tetraenoic acid (steer RIDON acid) [18:4, omega3], 11, 14, and 17-eicosatrienoic acid (******- alpha-linoleic acid) -- [20:3, omega3]. 8, 11, 14, 17-eicosatetraenoic acid [20:4, omega3], 5, 8, 11, 14, 17-eicosapentaenoic acid [20:5, omega3], 7, 10, 13, 16, 19-docosapentaenoic acid [22:5, omega3], 4, 7, 10, 13, 16, and 19- docosahexaenoic acid [22:6, omega3] can be mentioned.

[0033]omega6 system -- as unsaturated fatty acid -- 9 and 12-octadecadienoic acid [(linolic acid)] -- [18:2, omega6]. 6, 9, 12-octadecatrienoic acid (gamma- linolenic acid) [18:3, omega6], 8, 11, 14-eicosatrienoic acid (******- gamma-linolenic acid) [20:3, omega6], 5, 8, 11, 14-eicosatetraenoic acid (arachidonic acid) [20:4, omega6], 7, 10, 13, 16-docosatetraenoic acid [22:4, omega6], 4, 7, 10, 13, 16, and -docosapentaenoic acid [22:3, omega6] can be mentioned. [20:3, omega9] 11- eicosatrienoic-acid (mead acid) omega9 system -- as unsaturated fatty acid -- 6, 9- octadecadienoic acid [18:2, omega9], 8, 11-eicosadienoic acid [20:2, omega9], 5, and 8 -- it can mention. An acyl group may be hydroxylation, epoxidation, or an acyl group by which hydroxy epoxidation was carried out. The fatty acid which constitutes the 2nd place of new triglyceride of this invention consists of fatty acid of the carbon numbers 16-18. For example, pulmitic acid (16:0) and stearic acid (18:0) can be mentioned.

[Translation done.]

EXAMPLE

[Example]Next, an example explains this invention still more concretely. In this example, the following cable addresses show fatty acid and triglyceride for convenience. First, the following are used for the single-character cable address showing fatty acid. 8: Caprylic acid, P:pulmitic acid, A:arachidonic acid, M:mead acid, D : docosahexaenoic acid. Next, it writes by three characters with the single-character cable address showing the fatty acid which has combined triglyceride with the 1st place, the single-character cable address showing the fatty acid combined with the 2nd place, and the single-character cable address showing the fatty acid combined with the 3rd place. Therefore, the structure of triglyceride is written like the following example. Example: 8P8 (triglyceride which caprylic acid combined with caprylic acid at the 1st place, and combined with the 2nd place at pulmitic acid and the 3rd place)

[0035]1:2 (wt/wt) mixture of example 1. tripalmitin (PPP) and caprylic acid is used as a substrate raw material, The reaction mixture which turns into 10.5 g of substrate mixture from 1.2g of fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) is put into a vial bottle with a screw cap, It incubated shaking at 50 ** for 48 hours (a part for 140 times/). After the reaction, it left only immobilized enzyme, reaction mixture was exchanged for new substrate mixture, and it reacted under the same conditions. The reaction was performed 4 times, carrying out repeated use of the immobilized enzyme, and each reaction mixture was collected.

[0036]A 70-ml 0.5N KOH solution (20% ethanol solution) was added to each reaction mixture (10.5g), the evaporator removed the solvent after extracting a glyceride fraction by 100 ml of hexane, and glyceride was collected. As a result of investigating a glyceride presentation by an IYATO loss can (made by YATORON), 8% of diglyceride was contained in the 1st glyceride, but the partial-glycerides content in glyceride of the 2nd henceforth was 1% or less. The fatty acid composition (mol %) of the 2-4th glyceride fractions was 45.1% of caprylic acid, and 54.9% of pulmitic acid.

[0037]In order to raise the replacement factor of caprylic acid, the 2-4th glyceride fractions were used as the raw material, and the ester interchange was carried out again. The glyceride 3.5g and the caprylic acid 7g which were prepared were added to ribozyme IM60 (1.2g) used for the above-mentioned reaction, and it reacted, shaking at 30 ** for 48 hours (the 5th time). After the reaction, reaction mixture was exchanged for a new substrate and it reacted under the same conditions (the 6th time). Hexane extraction recovered the glyceride fraction from the 5 or 6th reaction mixture (a total of four .8 g). The fatty acid composition (mol %) of the obtained glyceride was 64.2% of caprylic acid, and 35.8% of pulmitic acid. The result which the partial glycerides contained in this glyceride are the following 1%, and was analyzed with the ODS column

(Wakosil-II 3C18, 4.6x150 mm, and 2) by using acetone/acetonitrile (1:1, vol/vol) as an elution solvent, The purity of 8P8 was 93%.

[0038]8P8 (3.5g) and 7 g of arachidonic acid (90% of purity) which were obtained were used as the raw material, the ester exchange reaction was performed at 30 ** ribozyme IM60 used for the above-mentioned reaction for 48 hours (the 7th time), hexane extraction of the reaction mixture after a reaction was carried out under alkali conditions, and the glyceride fraction (4.8g) was obtained. When the fatty acid composition of glyceride was analyzed, caprylic acid, pulmitic acid, gamma- linolenic acid, and arachidonic acid were 38.5, 23.1, 2.4, and 34.0-mol %, respectively. The result of having carried out fractionation of this glyceride with the high performance chromatography using an ODS column (SH-345-5, product made from 20 x 500mm YMC) by using acetone/acetonitrile (1:1, vol/vol) as an elution solvent, 8PA and 0.72 or 0.44g of APA were obtained, respectively.

[0039]It reacted on a scale of 100 times of the method indicated in the example 2. example 1, 8P8 was prepared, and it was used as a raw material. Lipase (Tanabe Seiyaku Co., Ltd. make; TARIPAZE) of Rhizopus delemar (Rhizopusdelemar) was fixed in ceramic carrier SM-10 (made by NGK Insulators, Ltd.) according to J. Ferment. Bioeng., 81, and 299-303 (1996). Soybean oil of water saturation after filling up a column with the immobilized enzyme 10g (31, 000 U/g): 100-ml sink immobilized enzyme was activated for caprylic acid 1:2 (wt/wt) mixed liquor by 30 ** and rate-of-flow 3 ml/hr.

[0040]Subsequently, after pouring 50 ml of soybean oil which is not adding water and removing superfluous water, the ester exchange reaction was performed, passing 1:4 (wt/wt) mixture of 8P8 and arachidonic acid ethyl ester (90% of purity) on the same conditions. After distilling 100 g of reaction mixture under the high vacuum and collecting glyceride fractions as residue, according to Example 1, hexane extraction was carried out under alkali conditions. The evaporator removed the solvent and 35.7 g hexane extractable material was obtained. It was 91:9 when the composition ratio of triglyceride and fatty acid ester which are contained in this hexane extractable material was analyzed by the IYATO loss can. As a result of analyzing fatty acid composition, they are caprylic acid, pulmitic acid, gamma- linolenic acid, and ******-. gamma- linolenic acid and arachidonic acid were 24.4, 34.5, 1.5, 2.6, and 37.0-mol %, respectively.

[0041]In order to remove the superfluous water contained in the fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) used in example 3. example 1, This immobilized enzyme 12g and SUNTGA - The cocktail which consists of 25 (made by Suntory) 60g is put into a 100-ml vial bottle with a screw cap, and it was made to react, shaking at 30 ** for 48 hours (the 1st time). It left only immobilized enzyme to the reactor, and after adding 8P8 (12g) and 48 g of mead acid ethyl ester (90% of purity) which were created in Example 2 and carrying out a nitrogen purge enough, the ester exchange reaction was performed, shaking at 30 ** for 72 hours (the 2 or 3rd time).

[0042]After the reaction, 100 g of them including the 2nd time and the 3rd cocktail was high vacuum distilled like Example 2, and glyceride fractions were collected as residue. Subsequently, after carrying out hexane extraction under alkali conditions according to Example 1, the evaporator removed hexane and a 24.1-g glyceride fraction was obtained. It was 92:8 when the composition ratio of triglyceride and fatty acid ester which are contained in this was analyzed by the IYATO loss can. High performance chromatography was performed according to Example 1, and MPM was 12.0% about a fixed quantity of fatty acid ester and each triglyceride ingredients from the peak area of a differential refractometer at the bottom and the time. Caprylic acid, pulmitic acid, and the mead acid of the fatty acid composition of this fraction were 31.2, 35.7, and 33.1-

mol %, respectively.

[0043]In order to raise a transesterification rate, the ester interchange of the obtained ester interchange triglyceride was again carried out by mead acid ethyl ester. It reacted, having added the ester interchange triglyceride 12g and 48 g of mead acid ethyl ester to the above-mentioned immobilized enzyme, and shaking at 30 ** for 72 hours (the 4th time). It distilled after the reaction by the method which mentioned above 55 g of reaction mixture, and the glyceride fraction of 12.3 g was obtained. Caprylic acid, pulmitic acid, and the mead acid of the fatty acid composition of this fraction were 5.2, 38.6, and 56.1-mol %, respectively.

[0044]In order to remove the superfluous water contained in the fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) used in example 4. example 1, This immobilized enzyme 2g and SUNTGA - The cocktail which consists of 25 (made by Suntory) 10g is put into a 20-ml vial bottle with a screw cap, and it was made to react, shaking at 30 ** for 48 hours (the 1st time). 8P8 (12g) and SUNTGA which left only immobilized enzyme to the reactor and were created in Example 2 - After adding 8 g of fatty acid mixture produced by hydrolyzing 25 and carrying out a nitrogen purge enough, the ester exchange reaction was performed, shaking at 30 ** for 48 hours (the 2-5th time). The glyceride which carried out hexane extraction from the 2-5th cocktails was set after the reaction, and it was considered as the substrate of the ester exchange reaction for the second time.

[0045]They are the ester interchange triglyceride 2g and SUNTGA to the reactor containing the above-mentioned immobilized enzyme. - 10 g of fatty acid mixture of 25 origin is added, and it was made to react, shaking at 30 ** for 48 hours (the 6 or 7th time). The glyceride fraction was extracted from the 6 or 7th cocktail, and it was considered as the substrate of the ester exchange reaction of a third-time degree, and reacted similarly (the 8th time). Gas chromatography analyzed each fatty acid composition, the fatty acid composition which constitutes the triglyceride obtained by repeating an ester exchange reaction 3 times, and triglyceride, of the 1 or 3rd place and the 2nd place. This result is shown in Table 1.

[0046]

[Table 1]

表１（単位：モル％）

脂肪酸の種類	新規構造脂質		
	全体	1，3位	2位
8：0	9	9	2
16：0	34	6	96
18：1 (n-9)	11	16	0
18：2 (n-6)	15	22	1
18：3 (n-6)	2	3	1
20：3 (n-6)	1	3	0
20：4 (n-6)	15	23	0

[0047]8P8 and immobilized enzyme which were created in comparative example 1. example 2 were used as a raw material and a catalyst, respectively. The immobilized enzyme 2g, the soybean oil 4g, the caprylic acid 8g, and the water 0.5g were put into a 20-ml vial bottle, and immobilized enzyme was activated by incubating shaking at 30 ** for 24 hours. It left the activated enzyme in the reactor and the substrate, the arachidonic acid /8P8 which does not contain water in this (4:1, wt/wt), or arachidonic acid/PPP (4:1, wt/wt) was added, and it carried out, having shaken the former reaction at 30 ** and shaking the latter reaction at

50 **. The reaction compared the stability of immobilized enzyme repeatedly, exchanging reaction mixture for the substrate which will seemingly be new every 24 hours.

[0048]When PPP is used for a substrate and a reaction is repeated at 50 **, after using immobilized enzyme 7 times, the uptake quantity of arachidonic acid fell to 10% or less of the first uptake quantity (the uptake quantity of the 1st time and the 7th arachidonic acid is 47% and 3%, respectively). When 8P8 was used for a substrate and a reaction was repeated at 30 ** on the other hand, even if it used immobilized enzyme 50 times, the uptake quantity of arachidonic acid hardly changed (the uptake quantity of the 1st time and the 50th arachidonic acid is 41% and 38%, respectively).

[Translation done.]

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omega6 system -- as unsaturated fatty acid -- 9 and 12-octadecadienoic acid [(linolic acid)] -- [18:2, omega6]. 6, 9, 12-octadecatrienoic acid (gamma- linolenic acid) [18:3, omega6], 8, 11, 14-eicosatrienoic acid (******- gamma-linolenic acid) [20:3, omega6], 5, 8, 11, 14- eicosatetraenoic acid (arachidonic acid) [20:4, omega6], 7, 10, 13, 16-docosatetraenoic acid [22:4, omega6], 4, 7, 10, 13, 16, and -docosapentaenoic acid [22:5, omega6] can be mentioned. [20:3, omega9] 11- eicosatrienoic-acid (mead acid) omega9 system -- as unsaturated fatty acid -- 6, 9- octadecadienoic acid [18:2, omega9], 8, 11-eicosadienoic acid [20:2, omega9], 5, and 8 -- it can mention. An acyl group may be hydroxylation, epoxidation, or an acyl group by which hydroxy epoxidation was carried out.

The fatty acid which constitutes the 2nd place of new triglyceride of this invention consists of fatty acid of the carbon numbers 16-18. For example, pulmitic acid (16:0) and stearic acid (18:0) can be mentioned.

[Translation done.]